

## Establishment of a bovine leukemia virus-free dairy herd in Korea

Guk-Hyun Suh\*, Jeong-Chi Lee<sup>1</sup>, Chai-Yong Lee<sup>2</sup>, Tai-Young Hur, Dong-Soo Son, Byeong-Seog Ahn, Nam-Chul Kim, Chung-Gil Lee<sup>2</sup>

National Livestock Research Institute, Rural Development Administration, Cheonan 330-800, Korea

<sup>1</sup>Department of Veterinary Nurse and Pets Sciences, Seojeong College, Yangju 482-777, Korea

<sup>2</sup>College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea

In view of the high prevalence rate of bovine leukemia virus (BLV) infections in cattle over the entire country, a large dairy farm in Chungnam province was chosen and 'test and segregate' program was instituted. On July 1999, ELISA test was performed on 491 animals on the farm and only 163 cattle (139 adult cows, 18 female and 6 male calves) were BLV-seronegative. From February 2000 through April 2004, the seronegative group was placed in barns 1,500 to 2,000 m from seropositive group and thereafter tested at 3- to 5-month intervals by ELISA. Animals seroconverted in consecutive tests were removed from the seronegative group immediately after the detection of anti-BLV antibodies. The changes in management were aimed at preventing iatrogenic transfer of blood between cattle. Replacement heifers imported from other countries and calves born at the farm were repeatedly tested by ELISA, and only seronegative animals were introduced into the group. As of April 2004, there were 311 cattle in the BLV seronegative group of the farm. Twenty four cows of the initial 139 adult cows were seroconverted in 2000, and no seropositive animals were found since February 2001. Follow up of the group, from which all seropositive cattle were moved to a separate location, revealed no recurrence of BLV infection for three years. The approach in the present study might be valuable for Korean producers who would like to move toward a BLV-negative status.

**Key words:** bovine leukemia virus, dairy cattle, ELISA, test and segregate

### Introduction

Enzootic bovine leukosis (EBL) is a naturally occurring disease of adult cattle caused by the retrovirus, bovine

leukemia virus (BLV). It is well known that BLV infections are prevalent in cattle populations in many countries, and the prevalence of the infection in cattle herds can reach 60 to 90% [5]. However, only a fraction of the cattle that became infected with BLV develop lymphosarcoma and persistent lymphocytosis, a benign proliferation of lymphoid cells. The infection, therefore, is economically important because of the loss of export markets that require BLV-free cattle, the costs incurred to diagnose, the premature culling or death of cattle of valuable breeding stock as a result of lymphosarcoma, and the condemnation of carcasses at slaughter [3].

For many years, diagnosis of BLV infection in cattle was based only on hematologic method consisted of counting the leukocytes of a blood sample in a counting chamber and applying the classification key of Bendixon [1]. Although persistent lymphocytosis was indeed an indicator of BLV infection, hematologic examinations did not identify all infected cattle. And the BLV-infected animals without lymphocytosis provided a constant source of virus within a given population. Discovery of BLV associated with the disease led to studies on serological diagnosis. The advent of sensitive and specific serological tests for the detection of BLV infections in cattle has stimulated renewed interest in control and eradication programs in countries that have already made a considerable commitment to such programs and have already made some progress in control of the disease. Among serological tests, the agar-gel immunodiffusion (AGID) test had been widely used to detect infected animals [2,4-7,13]. Recently, the enzyme-linked immunosorbent assay (ELISA) has proven to be rapid and sensitive test suited for testing large numbers of samples and can be used on milk samples [6,12,20].

Serological surveys have shown that BLV is widespread in Korea. In the early 1980s, random surveys by AGID revealed that 30 to 35% of dairy cattle were infected with BLV, whereas 2 to 6% of beef cattle were infected [2,6]. In an extensive survey in the early 2000s by ELISA, 54.2% of dairy cattle and 86.8% of dairy herds were infected with BLV, whereas 0.14% of beef cattle were infected [18]. These results indicate that BLV infection rates in Korea has

\*Corresponding author

Tel: +82-41-580-3405; Fax: +82-41-580-3429

E-mail: ghsuh@rda.go.kr

increased continuously during the last two decades. In view of the high prevalence rate of BLV infections in cattle throughout the country, a BLV control program was instituted at a high-prevalence, Holstein-Friesian dairy herd in the present study.

## Materials and Methods

### Herd background

A large dairy farm in Chungnam province, consisting of 500 to 600 Holstein-Friesian cattle a year, was chosen. It raised its own replacements, with limited introduction of heifers from other countries. Neonatal feeding practices at the farm were described earlier [17]. ELISA (CHEKIT-Leucotest; Bommeli AG, Swiss) test for anti-BLV antibodies performed on all animals of the farm revealed point prevalence rates of 49.7% in 1998 and 66.8% in 1999 with 32.8% of seroconversion rate [19] in 1998, the prevalence increased with the age of cattle, whereas no such trend was found in 1999 (Table 1).

### Management procedures

As of July 1999, there were 163 BLV-seronegative cattle, which was 33.2% of all animals at the farm; they were 139 adult cows, 18 female and 6 male calves. Strict separation of BLV-seronegative group was practiced from February 2000 through April 2004. The BLV-seronegative group was placed in barns 1,500 to 2,000 m from seropositive group and thereafter tested at 3- to 5-month intervals by ELISA. Animals seroconverted in consecutive tests were removed from the seronegative group immediately after the detection of anti-BLV antibodies.

Blood samples were taken from all calves born at the farm before ingestion of colostrum. Transplacental transmission is known to be relatively infrequent [22]. However, in order to save calves born to cows in the BLV-seropositive group, they were removed from their dams before receiving colostrum, reared in complete isolation and fed colostrum and milk from BLV-seronegative cows [11,14,17]. All

calves were placed in individual hutches and tested twice at two-week intervals after birth. If they were found serologically negative, they were placed with the BLV-seronegative group, females for replacement and males for feeding experiment.

Different, sterile, disposable needles and syringes were used for each venipuncture, vaccination, and intramuscular antibiotic injection. A different plastic, disposable obstetrical sleeve was used to palpate each BLV-seronegative cow. All cows were bred by artificial insemination. Instruments used for tattooing and ear-tagging were disinfected after each use by cleansing with a quaternary ammonium solution [4].

### Replacements

Replacement heifers were imported from Canada in 2001 and America in 2003. All heifers were seronegative for anti-BLV antibodies on 2 consecutive tests before importation; they were tested during the quarantine after importation. After they arrived at the farm, they were kept separated for one month and tested before introduction into the seronegative group.

## Results

Results of 'test and segregate' program used in the present study are shown (Table 2). Twenty-four adult cows of the original seronegative group were found to be seropositive on three successive tests only during the first year after segregation; they were removed from the seronegative group immediately after the detection of anti-BLV antibodies. As of April 2004, there were 311 cattle in the BLV seronegative group of the farm. There have been no infected animals since February 2001. During the experimental period, a total of 374 calves (213 females and 161 males) born at the farm were introduced into the seronegative group; none of the calves born to cows in the BLV-seropositive group were found to be seropositive. In addition to the calves, 50 heifers imported from Canada in 2001 and 44 heifers from America in 2003 were also introduced into the group. During the same period, 173 adult cows were removed from the group; of those, 24 cows were due to seroconversion and the rest were for culling due to old age, decreased milk yield and disease etc.

A total of 147 calves (18 females and 129 males) were either sold or removed for feeding experiment from the group.

## Discussion

This was the first attempt in Korea to establish a bovine leukemia virus-free dairy herd, using ELISA test and segregate method. All calves born at the farm during the experimental period were found to be negative for BLV antibodies and introduced into the seronegative group.

**Table 1.** Point prevalence of bovine leukemia virus antibodies of Holstein cattle determined in 1998 and 1999

Age	1998		1999	
	No. of cattle tested	Prevalence %	No. of cattle tested	Prevalence %
0- 6 months	80	36.3	27	70.4
>6-12 months	62	21.0	44	61.4
>1-2 years	87	36.8	89	46.1
>2-3 years	83	66.3	79	51.9
>3-4 years	82	56.1	65	80.0
>4-5 years	59	50.8	79	81.0
>5 years	118	66.9	108	77.8
Total	571	49.7	491	66.8

**Table 2.** Establishment of a bovine leukemia virus-free Holstein dairy herd\*

Year		Results of ELISA test	
		No. of cattle tested	No. of cattle positive
2000	April	163	15
	July	188	5
	November	152 ( 4) <sup>†</sup>	4
2001	February	143 ( 1) <sup>‡</sup>	0
	July	177 (50) <sup>‡</sup>	0
	October	209 ( 4) <sup>‡</sup>	0
2002	March	240 ( 9) <sup>‡</sup>	0
	July	273 (20) <sup>‡</sup>	0
	October	273 ( 9) <sup>‡</sup>	0
2003	January	289 ( 8) <sup>‡</sup>	0
	April	248 ( 4) <sup>‡</sup>	0
	July	263 (44) <sup>‡</sup>	0
	October	285 ( 4) <sup>‡</sup>	0

\*In February 2000, 163 cows bovine leukemia virus (BLV) antibody negative were segregated.

<sup>†</sup>Number of calves in the parentheses (the female calves born to cows in the BLV-seropositive group) were included in the number of cattle tested. All calves born to both BLV antibody negative and positive cow groups since the segregation were tested for BLV antibodies at regular intervals twice at 2-week interval after birth and BLV antibody negative calves only were kept for replacement.

<sup>‡</sup>Number of heifers in the parentheses were included in the number of cattle tested. During the experiment, heifers were imported from Canada in 2001 and America in 2003. All heifers were tested for BLV antibodies before and after importation and used for replacements.

Replacement heifers imported from Canada and America were tested four times and accepted there. For more than three years there have been no infected animals in the segregated, seronegative group of the farm. The results clearly indicate that the program employed in the present study was successful in eradicating BLV infection from a high-prevalence, Holstein-Friesian dairy herd.

As the natural spread of the virus apparently occurs at a relatively low rate among susceptible cattle and the virus is spread by movement of infected animals from one herd to another and within a herd [10], attempts to eradicate BLV have been successful. For decades, three approaches to eradication have been used with the advent of sensitive and specific serological tests such as AGID [3,5,16,21]. In the present study, ELISA test was used for BLV antibodies, which was recently proved to be effective in BLV control program in Finland [9]. The results of the BLV control program in the present study were comparable to those of numerous other researchers [7,8,14,23], indicating ELISA test effective in screening cattle for BLV antibodies.

Herds with a lower seroprevalence of BLV infection, in general, required fewer tests to eliminate infected cattle [3]. The herd enrolled in the present study had a fairly high

(66.8%) prevalence rate, but seropositive cattle were eliminated after three ELISA tests; follow up of the seronegative group, from which all seropositive cattle were moved to a separate location, revealed no recurrence of BLV infection for three years, again indicating the effectiveness of ELISA test for BLV antibodies. The test was proven to be rapid and sensitive and can be used on milk samples [12]. In fact, the accuracy of ELISA test for BLV antibodies was demonstrated in the results of previous reports [2,6,18], in which BLV infection rates in beef cattle decreased dramatically with time. EBL is a notifiable disease, and 86.8% of dairy herds were found infected with BLV in Korea [18]. Because of the economic importance of EBL, attempts were made to eradicate the disease in many countries. Three approaches to eradication have been used; of those, 'test and slaughter' method creates varying degrees of economic hardship, depending on the prevalence of infection. And 'test and implement corrective management' method requires long-term commitment. The test and segregate method used in the present study has been used more frequently than other methods in many countries [3].

The approach in the present study might be valuable for Korean producers who would like to move toward a BLV-negative status but would be unwilling to remove a considerable number of animals from their herds in an all-out effort to eliminate BLV infections.

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